



## **Non-AIDS-defining deaths and immunodeficiency in the era of combination antiretroviral therapy.**

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**Abstract**

**Objective:** To assess whether immunodeficiency is associated with the most frequent non-AIDS-defining causes of death, in the era of combination antiretroviral therapy (cART).

**Design:** Observational multicenter cohorts.

**Methods:** 23 cohorts of adults with estimated dates of Human Immunodeficiency Virus (HIV) seroconversion. Patients were seroconverters followed within the cART era. Measurement were latest CD4; nadir CD4 and time spent with CD4 <350 cells/mm<sup>3</sup>. Outcomes were specific causes of death using a standardized classification.

**Results:** Among 9,858 patients (71,230 person-years follow-up), 597 died, 333 (55.7%) from non-AIDS-defining causes. Non-AIDS-defining infection, liver disease, non-AIDS-defining malignancy and cardiovascular disease (CVD) accounted for 53% of non-AIDS deaths. For each 100 cells/mm<sup>3</sup> increment in the latest CD4 count, we found a 64% (95%CI 58-69%) reduction in risk of death from AIDS-defining causes and significant reductions in death from non-AIDS infections (32, 18-44%), end-stage liver-disease (33, 18-46%), and non-AIDS malignancies (34, 21-45%). These risks were also associated with nadir CD4 while cART-naïve and duration of exposure to immunosuppression. No relationship between risk of death from CVD and CD4 count was found although there was a raised risk associated with elevated HIV RNA.

**Conclusions:** In the cART era, the most frequent non-AIDS-defining causes of death are associated with immunodeficiency, only CVD was associated with

high viral replication. Avoiding profound and mild immunodeficiency, through earlier initiation of cART, may impact on morbidity and mortality of HIV infected patients.

**Keywords (Mesh terms)**

- 1) Causes of death
- 2) Human Immunodeficiency Virus/AIDS
- 3) CD4 cell count
- 4) Antiretroviral therapy, highly active
- 5) Neoplasm
- 6) Hepatitis
- 7) Bacterial infection

## **Introduction**

With the widespread use of combination antiretroviral therapy (cART), mortality from the Human Immunodeficiency Virus (HIV) infection has decreased from around 10-12 to 1-3 per 100 person-years [1-6]. As a consequence of increased survival, non-AIDS defining conditions, specifically malignancies, end stage liver disease, cardiovascular disease, severe infections and kidney disease [7-9], now account for between 50 to 66% of deaths that occur.

There is growing evidence that the incidence of a variety of non-AIDS conditions may be associated with longer duration of immunodeficiency, even in cART treated patients [10-16]. Whether HIV infection itself explains these emerging causes of non-AIDS defining mortality through exposure to uncontrolled viral replication or to persistent immune suppression might have important implications in terms of the optimal timing of antiretroviral treatment.

To date, most studies investigating cause-specific mortality in HIV infection have used data from prevalent cohorts and have restricted themselves to examining the current level of immunodeficiency, as measured by the latest CD4 cell count. However, the latest CD4 cell count may be a consequence of the event rather than its cause, if the event (or its treatment) has an immunodepressive effect, e.g. cancer and chemotherapy. Other measurements of immunodeficiency, such as the nadir CD4 cell count or the time spent with a low CD4 cell count, may better reflect an individual's prior

exposure to immunodeficiency, as they reveal, respectively, the most profound alteration to the immune system and the length of exposure to this condition. The impact of such markers may be more reliably assessed in the long-term follow-up of seroconverters, the vast majority of whom are followed prospectively shortly after seroconversion. In cohorts of such individuals, survival can be described from a similar date of origin for all patients and the measure of association between time-dependent markers and death will be less subject to bias [17].

We used data from CASCADE, a large multicenter collaboration of cohorts of HIV-infected individuals with well-estimated date of HIV seroconversion to investigate the relationships between non AIDS-defining deaths and HIV-associated immunodeficiency in the era of cART. We also investigated whether this relationship was consistent when using different approaches to account for exposure to immunodeficiency.



## **Methods**

CASCADE (Concerted Action on SeroConversion to AIDS and Death in Europe) is a collaboration between the investigators of 23 cohorts in Europe, Canada and Australia who collect data on HIV-1 infected individuals for whom it is possible to reliably determine the date of HIV seroconversion [18]. The CASCADE database is updated annually and the current analysis is based on data merged in September 2006.

### **Study population**

Patients older than 16 years at the time of seroconversion with available follow-up since 1<sup>st</sup> January 1996, the year in which cART became widely available, were eligible for inclusion in this current analysis. In addition, we selected individuals with at least two CD4 and HIV RNA measurements available more than six months after seroconversion. If treated with cART, patients were also required to have markers measured in the six months before and the 12 months after treatment initiation (regardless of CD4 count availability, all patients dying in the first year were retained in the analysis).

### **Causes of death**

Classification of specific causes of death was based on the 1993 clinical definition of AIDS from the Centers for Disease Control [19], USA (i.e other clinical conditions were considered to be non AIDS-defining) and the International Classification of Diseases 10<sup>th</sup> Revision (ICD-10). Furthermore

the “Coding of Death in HIV” (CoDe) classification system was used to standardize causes of death reported by cohorts ([www.cphiv.dk/CoDe](http://www.cphiv.dk/CoDe)).

### Statistical Methods

Analyses were performed using the SAS<sup>®</sup> software v9 (SAS institute, Cary, NC). The cumulative incidence of each specific cause of death was estimated within a competing risk framework [20]. Person-years of follow-up (PYFU) were calculated from date of enrolment in the cohorts. The multivariate analysis used the cause-specific hazard in multivariable Cox proportional hazards models [21, 22], allowing the use of time-varying covariates. In Cox models, patients dying from causes other than those specifically studied were right-censored. Follow-up time was considered from the date of seroconversion to outcome or censoring date, with patients’ follow-up being right-censored on the last date known to be alive. The analyses allowed for late entry into the risk set in order to take into account the lag time between the estimated date of seroconversion and the latest of date of enrolment into the constituent CASCADE cohort, and dates of first CD4 count and HIV RNA assessment. Using this left truncation approach, we were able to adjust for possible bias in estimates due to the effect of survivorship. All analyses were stratified by cohort in order to take potential heterogeneity into account.

We fitted separate multivariable models for the 10 most frequent causes of death and modelled the intensity and duration of immunodeficiency with the following time-dependent variables: (i) latest CD4 count; (ii) nadir CD4 cell count over the entire follow-up period to date; (iii) nadir CD4 cell count while

cART-naïve (nadir value over the entire follow-up period to date was used for those not yet starting cART); and (iv) cumulative time spent with a CD4 cell count  $<350$  cells/mm<sup>3</sup>. Distinct models were developed for these four measures of immunodeficiency since they were highly correlated.

Given that our cohort populations were frequently followed-up, CD4 count and HIV RNA values over follow-up (treated as time varying covariates) were each estimated using the moving average of the previous two reported measurements in order to minimize the impact of measurement error or erroneous data. The cumulative time spent at CD4 counts below 350 cells/mm<sup>3</sup> was assessed by allocating time on the basis of actual CD4 cell counts and was updated in the model each time a new CD4 count became available.

Latest and nadir CD4 counts were categorized according to clinically relevant cut-offs, i.e.  $<50$ , 50 to 199, 200 to 349 and  $\geq 350$  cells/mm<sup>3</sup>. Analyses were adjusted for other characteristics known to be associated with survival. Log<sub>10</sub> HIV RNA level, performed at the same time as the CD4 cell count, was categorised as  $<5$  vs.  $\geq 5$  log<sub>10</sub> copies/ml, as a marker of subsequent lymphocyte activation or endothelial activation to high HIV RNA replication. The choice of cut-off for time-dependent HIV RNA was based on the fact that HIV RNA distribution did not suggest a log-linear relationship, and the large number of patients who became undetectable during follow-up militated against its use as a continuous variable. We also adjusted for sex, age at seroconversion (which did not satisfy the assumption for log-linear risk association and was subsequently categorized as  $<35$  vs.  $\geq 35$  years, to

account for the increased risk of cancer and cardiovascular disease in older patients, as well as late entry into the risk set), exposure category (injecting drug use (IDU) vs. other modes), baseline hepatitis C (HCV) status. As treatments may also influence survival from some causes that are not AIDS-defining [13, 23], we adjusted for initiation of cART as a time-dependent covariate. The analysis was performed on an intention-to-continue cART basis, ie. we ignored subsequent treatment changes. In a secondary analysis, we incorporated an interaction term between HIV RNA level and cART status (treated or not treated). Thus all analyses presented in this report are adjusted for CD4 cell count, HIV RNA level, age, sex, exposure category, HCV status and first line cART.

In addition, we performed sensitivity analyses for cardiovascular and non-AIDS defining malignancy deaths, using the CD4 count lagged by six months (i.e. the values incorporated into the model were all measured at least six months before death). This latter approach was taken to better disentangle the role of this marker as the cause, rather than the consequence, of the evolving condition leading to death.

## Results

Among 15,032 patients in CASCADE older than 16 years with available follow-up after 1<sup>st</sup> January 1996, 13,145 had CD4 cell count and HIV RNA measurements available at least once from six months after seroconversion (among whom 69% started cART during follow up). Of these, 2,484 treated patients did not have markers measured in the six-month period before or the twelve-month period after cART initiation, and a further 803 had fewer than two measurements of each marker. Among the 5,174 excluded patients (33,532 PYFU), 32% had seroconverted after 1996 and 689 deaths occurred (50% from AIDS-defining causes). Finally, 9,858 patients were included in our analysis. The patients were mostly male (77%) and had acquired HIV through sex between men (54%), between men and women (26%), or through injecting drug use (15%). Where Hepatitis C serostatus was known at seroconversion (79%), one fifth (21%) of patients were co-infected. The median date of HIV seroconversion was July 1996 and median age at seroconversion was 30 years (interquartile range: IQR, 25–36). The median time between the date of seroconversion and date of entry into the cohort was 6.6 months (IQR, 1.5–14.5).

Per patient, a median of 12 measurements of HIV RNA (IQR, 5–23) and 15 CD4 cell counts (IQR, 6–29) were available. For patients who died, the median lag times between the last available CD4 count or HIV RNA determination and death were 2.5 months (IQR, 1.0–5.6) and 3.0 months (IQR, 1.2–6.4), respectively.

### **Specific causes of death**

During 71,230 PYFU, 597 patients died. The 5-year and 10-year cumulative incidences for all-cause mortality were 1.5% and 5.7%, respectively. Most deaths were from non AIDS-defining causes (333 of 597), 178 (53%) of which were due to non-AIDS-defining infections (50 deaths), end-stage liver disease (46 deaths), non-AIDS-defining malignancies (46 deaths) or cardiovascular disease (36 deaths), (table 1). The 5-year cumulative incidence of these four specific causes of death were 0.15%, 0.03%, 0.06% and 0.05% and at 10 years it was 0.51%, 0.23%, 0.51% and 0.26%, respectively.

### **Antiretroviral treatment**

Throughout follow-up, 6,530 patients (66.3%) started cART, 743 (7.5%) received only mono- or dual therapy while 2,585 (26.2%) remained untreated. Among the 6,530 patients who started cART, 3,884 (59.5%) were antiretroviral naïve when they started. The median time between seroconversion and cART initiation was 3.8 years (IQR, 1.3–7.3). Among those who started cART, the median CD4 cell count and HIV RNA level at treatment initiation were 324 cells/mm<sup>3</sup> (IQR, 211–475) and 4.6 log<sub>10</sub> copies/ml (IQR, 3.7–5.2), respectively. Median CD4 cell count and HIV RNA levels measured, on average, 6 months (4 to 8 months) after cART initiation were 451 cells/mm<sup>3</sup> [302–627] and 2.30 log<sub>10</sub> copies/ml [1.69–2.95], respectively. At 12 months (8 to 16 months), they were 480 cells/mm<sup>3</sup> [330–670] and 2.30 log<sub>10</sub> copies/ml [1.96–3.22].

cART regimens were mostly PI-based (60%, including 9% with boosted PI-based regimens) or non-nucleoside reverse transcriptase inhibitor-based (30%). Nucleoside reverse transcriptase inhibitor-based regimens alone accounted for 10% of initial regimens.

### **Relationship between markers of immunodeficiency and specific causes of death**

For each 100 cell/mm<sup>3</sup> increment in the latest CD4 count, there was a 32% reduction in the risk of all cause mortality (95% CI, 28-35%), after adjustment for the latest HIV RNA level, age, sex, exposure category, HCV serostatus and initiation of cART treatment (Figure 1). When examining specific causes of death, a two-thirds reduction in mortality per each 100 cell/mm<sup>3</sup> increment in CD4 lymphocytes was observed for AIDS-defining causes (64%, 58-69%). One-third reductions in risk were observed for non-AIDS infections (32%, 18-44%), end-stage liver disease (33%, 18-46%), and non-AIDS malignancies (34%, 21-45%). Reduction in mortality from all other or unknown causes, with the exception of violent deaths (including suicide), was also associated with an increase in the latest CD4 count. The univariate relationship between the latest CD4 cell count and cardiovascular deaths (17% reduction, 2-29) was no longer significant after adjustment for latest HIV RNA level and the other covariates (14%, 95% CI= 27% decrease to 2% increase).

There was a clear gradient effect between all markers of immunosuppression: the latest CD4 cell count, nadir CD4 cell count over the

entire follow up or while cART-naïve, and the cumulative time spent with CD4 cells  $<350/\text{mm}^3$  and mortality from AIDS-defining causes, non AIDS-defining infections and respiratory diseases (Tables 2 and 3). There was a clear gradient effect of latest and nadir CD4 count (measured throughout follow-up) on mortality from end-stage liver disease as well as substance abuse. A similar trend was apparent for deaths from non-AIDS malignancies, although no association was found between risk from these deaths and duration of time with a CD4 count  $<350$  cells/ $\text{mm}^3$ . Age at seroconversion was associated with a higher mortality from each specific cause of death. Patients older than 35 years at seroconversion had about twice the risk of dying from AIDS (HR=2.22 [95% CI, 1.50-3.28]), non AIDS infection (2.04 [95% CI, 1.04-4.10]) and liver disease (2.28 [95% CI, 1.08-4.83]) than younger patients. The effect of age was even stronger for mortality due to non-AIDS cancer: 3.29 (95% CI, 1.76-6.15) and cardio-vascular diseases: 3.61 (95% CI, 1.69-7.72). There was no association between mortality due to cardiovascular causes and any of the markers of immunosuppression considered, although an association was apparent with the latest HIV RNA level. Mortality due to violence (including suicide) were not associated with any marker of immunodeficiency, or with the latest HIV RNA level.

For the analysis of cardiovascular and non-AIDS defining malignancy deaths, we found hazard ratios within the same range when we considered the latest CD4 count measured at least six months before death (instead of the latest value available immediately preceding death).



Individuals with a high HIV RNA level, whether treated or not, had a significantly higher mortality from AIDS-defining and non-AIDS infectious causes (Table 4) even after adjusting for latest CD4 count. An association between liver disease mortality and a high HIV RNA level was only apparent among patients not on cART. Deaths from non-AIDS malignancies were not associated with HIV RNA level, whether the patient was receiving cART or not. Finally, deaths from cardiovascular causes were clearly associated with a higher HIV RNA level, regardless of cART (HR=3.86, 1.57-9.51).

We did not correct type I error inflation for multiple comparisons, but most associations were highly significant ( $p < 0.001$ ) and would allow for more than 50 comparisons, assuming an overall type I error of 0.05 in a conservative Bonferroni correction.

## Discussion

In this large collaboration of HIV seroconverter cohorts, non-AIDS defining causes contribute to more than half of deaths especially serious non-AIDS-defining infections, malignancies, end-stage liver, cardiovascular and respiratory disease. We provide evidence for an association between these causes of death and several markers of immunodeficiency, with the exception of cardiovascular deaths which were, however, associated with higher levels of viral replication.

Our analysis confirms that latest CD4 cell count is associated with a higher mortality from conditions not traditionally defined as opportunistic, i.e liver diseases, non AIDS-infections, respiratory diseases, as well as non AIDS-malignancies, even when CD4 count measured at least six months before death was considered. In addition, we found a consistent and gradually increasing association between earlier markers of immunodeficiency, such as the nadir CD4 count, or duration of exposure to immunodeficiency, such as time spent with  $CD4 < 350/mm^3$  and these specific causes of non-AIDS defining deaths. Our analysis does not indicate a particular advantage of either CD4 cell marker compared to the latest CD4 cell count for non-AIDS related outcomes. However, the consistent finding of a raised risk of non-AIDS defining death and cumulative time spent below  $CD4 < 350/mm^3$  reveals important clinically-relevant information and provides a strong argument that HIV-infected patients may benefit from early initiation of antiretroviral treatment to reduce the risk of AIDS and non AIDS-related causes of death [24].

In this study, high HIV RNA, but not CD4 cell markers, was associated with a higher risk of cardiovascular disease death. High HIV RNA level may be considered as a surrogate marker of HIV-related endothelial inflammatory activation, such as Interleukin -6 or ultra-sensitive C-reactive protein, that may be promoted through various mechanisms [25].

Our study shows in particular a specific association of immune depletion and mortality from liver and cancer which may be additionally related to the result of ageing [26], HCV and hepatitis B virus (HBV) infections, tobacco, alcohol, and injecting drug use [27-29] as well as to toxicities from antiretrovirals [30].

Our study also shows that liver disease is a frequent cause of death in the cART era, now that patients are surviving to experience this competing cause of death. Indeed, immunosuppression is associated with more rapid progression to liver fibrosis and cirrhosis [13, 31]. Mortality rates associated with end-stage liver disease may rise even further in the future due to the ageing of the HIV population, co-infections with viral hepatitis B and C, and long-term cART-related hepatotoxicities [32].

A recent meta-analysis comparing the incidence of 28 types of cancer between HIV-infected patients, organ transplant recipients, and the general population, suggested that immunodeficiency was the main cause of cancer development [33]. Although HIV is usually not considered as an oncogenic virus, HIV infection may contribute to carcinogenesis by persistent inflammation mediated by cytokines especially as immunosuppression

provides the immunologic background that favours the development of non-AIDS-defining cancer [16, 34] and leads to a reduced ability to the control of oncogenic viruses [35].

Studies exploring the relationship between immunodeficiency and non-AIDS-defining causes of death, however, have provided limited or biased evidence because they were based on small cohorts [36] or used models based on CD4 cell measurements immediately preceding death. This study confirms findings from prevalent cohorts reporting that the latest CD4 cell count and time since seroconversion were associated with deaths from both pre-AIDS [37, 38] and non-AIDS causes. In addition, results from the SMART trial clearly implicate immunodeficiency with the occurrence of several severe non-AIDS events, including major cardiovascular events, end-stage liver disease, malignancies and kidney complications [39, 40].

Seroconverter cohorts, such as CASCADE, are better suited than prevalent HIV populations for studying the determinants of survival. As seroprevalent cohorts are recruited at various times after seroconversion with unknown HIV infection duration, early deaths might be missed leading to an underestimation of death rates and biased estimates of prognostic factors. In our analyses, the long duration of follow-up acquired for this population of seroconverters allowed us to assess four different approaches to model 'exposure' to immunosuppression, which ensured that immunosuppression had preceded death, rather than simply reflected the effects of terminal

disease. Nevertheless, as all patients were under follow-up shortly after seroconversion, and based on inclusion criteria they may represent a selected population with earlier HIV diagnosis, closer follow-up, optimal HIV management and favourable outcome, as compared to a recent publication from the same collaboration [1]. In addition, patients started cART with a median CD4+ above 320/mm<sup>3</sup>, substantially higher than the level seen in many seroprevalent cohorts [41, 42], reflecting the fact that patients were managed in an optimal setting in the context of early HIV diagnosis. However, although the overall mortality and the cumulative incidence of specific causes of death might under-estimate that in the wider HIV-infected population [13, 15, 43-46], it is unlikely that the association between immunodeficiency and deaths from different causes would be greatly affected by this selection. Finally, the patients selected for this analysis might better reflect patients that are currently managed and followed-up by practitioners, as attested by a larger proportion of HIV acquisition in the more recent periods.

The quality of our database depended on the accuracy of recording of cause of death in each cohort, with 82% of causes that could be classified. Deaths were standardized using the Coding of Causes of Death classification ([www.cphiv.dk/CoDe](http://www.cphiv.dk/CoDe)) [11], which ensured homogeneous coding for the reason of death across cohorts. Nevertheless, we did not apply the central adjudication process as in controlled trials, due to the fact our categories of deaths were large and therefore misclassification unlikely. However, each cohort uses its own methods to collect source data and the process for

selecting and validate an underlying cause of death may differ across cohorts, explaining that 18% of causes of death remained unknown. Anyway, in such a situation, the proportion of deaths attributed to AIDS have likely been overestimated as patients with different causes of death might have been more likely to be classified as AIDS-defining when their current CD4 cell count was low. As a result, this misclassification may lead to an underestimation of the relationship between non AIDS-defining causes of death and immunodeficiency. Thus, we do not believe that this bias could explain the strong associations with immunodeficiency that we observed.

Treatment adherence was not available in the CASCADE dataset, however any clinically relevant effect would be captured by our primary exposure variables (HIV RNA and CD4 cell count). Besides, traditional risk factors for specific non-AIDS-defining causes of death, such as tobacco consumption, could not be included as they are currently not pooled in CASCADE. It is difficult, however, to see how many of these factors would confound any relationship with immunodeficiency.

In conclusion, our results add to the growing body of evidence on the association of immunodepletion and important non-AIDS related morbidity [47] and mortality and underline the need for continued large scale collaborative HIV cohort collaborations. Our results also plead for the use of non-AIDS defining death and morbidities as endpoints in clinical trials evaluating antiretrovirals. As HIV-infected patients in industrialized countries

have higher life style related risks factors (e.g. alcohol and tobacco consumption) compared to the general population [29], programmes for aggressive management of life style related cardiovascular risk factors are paramount [48, 49]. The burden of infections points to the need for harm reduction programs especially for injecting drug users who are at higher risk of dying from non-AIDS-defining causes [50] in addition to improved management strategies for the treatment of chronic HCV and HBV infections. Most importantly, our data provide additional arguments for HIV screening programs of at-risk populations for the early detection and treatment of HIV infection.

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### **Contributors**

Geneviève Chêne, Benoît Marin, Rodolphe Thiébaud and Virginie Rondeau wrote the first draft of the study protocol (scientific hypothesis, objective, choice of study design and statistical methods, eligibility criteria). All members of the steering

committee contributed to the final version of the protocol. Benoît Marin, Rodolphe Thiébaut and Virginie Rondeau performed statistical analyses. All authors contributed to interpretation of the results. Geneviève Chêne and Benoît Marin wrote the first draft of the manuscript. All authors contributed to the final text. Caroline Sabin, Kholoud Porter, A Sarah Walker, Heiner C Bucher, Rodolphe Thiébaut, Geneviève Chêne and Benoît Marin contributed to the editing.

**Conflict of interest statement**

Geneviève Chêne has received travel consultancy fees and honoraria from Boehringer Ingelheim, Roche and Gilead Sciences.



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Table 1. Markers of immunodeficiency according to specific causes of death, CASCADE collaboration, 1996-2006.

	Distribution		Time from seroconversion* (years)		Nadir prior to starting cART		CD4 count (/mm <sup>3</sup> ) Nadir over the entire follow-up		Latest marker <sup>†</sup>	
	Number	%	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Patients thought to remain alive	9261	-	7.6	(3.4 - 11.9)	304	(204 - 432)	281	(180 - 413)	498	(351 - 682)
Causes of death										
AIDS-defining death	158	26.5	8.6	(6.0 - 13.2)	42	(10 - 170)	24	(7 - 68)	45	(10 - 145)
Non AIDS-defining infection	50	8.4	8.4	(5.2 - 14.5)	115	(39 - 240)	79	(28 - 188)	189	(32 - 397)
Liver disease <sup>‡</sup>	46	7.7	12.2	(9.8 - 15.2)	110	(53 - 215)	95	(39 - 200)	159	(96 - 308)
Non AIDS-defining cancer	46	7.7	8.4	(7.0 - 12.6)	211	(97 - 308)	159	(76 - 254)	236	(134 - 406)
Cardiovascular disease	36	6.0	11.0	(7.1 - 14.8)	170	(92 - 268)	149	(58 - 253)	270	(116 - 501)
Suicide	38	6.4	7.7	(4.3 - 10.7)	298	(180 - 414)	291	(145 - 397)	513	(327 - 831)
Substance abuse <sup>#</sup>	34	5.7	6.2	(4.6 - 9.7)	182	(81 - 403)	157	(46 - 388)	292	(100 - 483)
Violent cause	20	3.3	7.5	(3.8 - 12.9)	337	(269 - 498)	318	(165 - 430)	629	(377 - 781)
Respiratory disease	20	3.3	8.3	(6.6 - 10.6)	140	(15 - 258)	20	(5 - 171)	25	(5 - 356)
Other medical cause	43	7.2	10.4	(7.0 - 12.3)	170	(88 - 306)	126	(42 - 239)	252	(88 - 455)
Unknown cause	106	17.8	9.8	(5.8 - 13.0)	163	(80 - 274)	120	(47 - 252)	250	(103 - 430)
Dead patients overall	597	100.0	9.0	(5.8 - 12.7)	150	(40 - 269)	100	(20 - 240)	190	(49 - 420)

\* Time from seroconversion is time from date of seroconversion to date of death or censoring date (for those who remained alive)

† Latest marker is latest measurement of the CD4 count at the time of death or at last follow-up (for those who remained alive)

‡ Liver disease : death due to hepatitis B or C or end-stage liver failure

# Substance abuse : death due to injecting drugs, acute intoxication, alcohol

IQR : interquartile range

Table 2. Adjusted cause-specific hazard ratios of progression to death associated with latest CD4 cell count (first column), nadir CD4 cell count over the entire follow up (second column) and nadir CD4 cell count while cART-naïve (third column), CASCADE collaboration, 1996-2006.

		Latest CD4			Nadir CD4 over the entire follow-up			Nadir while cART-naïve		
		Adj HR*	95% CI	p-value	Adj HR*	95% CI	p-value	Adj HR*	95% CI	p-value
AIDS death	CD4 count (/mm <sup>3</sup> )			< 0.0001			< 0.0001			< 0.0001
	349-200 vs ≥350	4.70	(2.46 - 8.99)		2.31	(1.06 - 5.02)		1.38	(0.73 - 2.64)	
	199-50 vs ≥350	30.15	(16.79 - 54.15)		12.41	(6.13 - 25.14)		4.95	(2.74 - 8.94)	
	<50 vs ≥350	157.58	(84.72 - 293.10)		67.92	(33.05 - 139.57)		15.66	(8.81 - 27.82)	
non AIDS infection	HIV RNA (log <sub>10</sub> /ml) ≥5 vs <5	2.21	(1.50 - 3.28)	< 0.0001	4.40	(3.03 - 6.41)	< 0.0001	3.14	(2.17 - 4.55)	< 0.0001
	CD4 count (/mm <sup>3</sup> )			< 0.0001			< 0.0001			0.003
	349-200 vs ≥350	0.95	(0.36 - 2.48)		0.88	(0.30 - 2.55)		1.30	(0.53 - 3.22)	
	199-50 vs ≥350	5.66	(2.49 - 12.86)		5.20	(2.09 - 12.92)		3.98	(1.67 - 9.47)	
Liver disease	<50 vs ≥350	16.02	(5.91 - 43.45)		11.57	(3.99 - 33.47)		3.78	(1.39 - 10.26)	
	HIV RNA (log <sub>10</sub> /ml) ≥5 vs <5	6.23	(3.14 - 12.38)	< 0.0001	9.24	(4.87 - 17.50)	< 0.0001	5.90	(3.18 - 10.97)	< 0.0001
	CD4 count (/mm <sup>3</sup> )			< 0.0001			0.01			0.21
	349-200 vs ≥350	2.18	(0.88 - 5.36)		2.59	(0.80 - 8.39)		1.71	(0.67 - 4.38)	
non AIDS cancer	199-50 vs ≥350	7.07	(3.02 - 16.54)		4.00	(1.22 - 13.09)		1.52	(0.56 - 4.15)	
	<50 vs ≥350	6.57	(2.00 - 21.6)		7.99	(2.19 - 29.15)		2.96	(1.02 - 8.61)	
	HIV RNA (log <sub>10</sub> /ml) ≥5 vs <5	1.31	(0.55 - 3.12)	0.54	1.85	(0.80 - 4.27)	0.15	2.48	(1.21 - 5.07)	0.012
	CD4 count (/mm <sup>3</sup> )			< 0.0001			0.015			0.02
Cardiovascular disease	349-200 vs ≥350	2.74	(1.31 - 5.74)		1.87	(0.81 - 4.35)		2.24	(0.94 - 5.37)	
	199-50 vs ≥350	5.42	(2.41 - 12.21)		1.92	(0.74 - 4.97)		1.92	(0.69 - 5.38)	
	<50 vs ≥350	18.70	(5.91 - 60.46)		5.79	(1.94 - 17.22)		4.70	(1.75 - 12.61)	
	HIV RNA (log <sub>10</sub> /ml) ≥5 vs <5	1.10	(0.39 - 3.14)	0.85	2.17	(0.82 - 5.72)	0.12	0.52	(0.16 - 1.74)	0.28
Violent cause†	CD4 count (/mm <sup>3</sup> )			0.14			0.66			0.38
	349-200 vs ≥350	1.15	(0.51 - 2.63)		1.43	(0.54 - 3.79)		1.69	(0.86 - 9.18)	
	199-50 vs ≥350	0.89	(0.28 - 2.82)		1.21	(0.41 - 3.59)		1.55	(0.55 - 4.37)	
	<50 vs ≥350	4.15	(1.14 - 15.17)		2.13	(0.57 - 7.99)		2.82	(0.64 - 4.43)	
Substance abuse	HIV RNA (log <sub>10</sub> /ml) ≥5 vs <5	3.86	(1.57 - 9.51)	0.003	4.27	(1.84 - 9.93)	0.0007	1.68	(0.74 - 3.81)	0.22
	CD4 count (/mm <sup>3</sup> )			0.59			0.24			0.27
	349-200 vs ≥350	0.77	(0.38 - 1.58)		0.49	(0.25 - 0.99)		0.57	(0.29 - 1.13)	
	199-50 vs ≥350	0.61	(0.20 - 1.87)		0.73	(0.33 - 1.61)		0.49	(0.20 - 1.22)	
Respiratory disease	<50 vs ≥350	0.61	(0.20 - 1.87)		1.01	(0.31 - 3.31)		0.85	(0.36 - 1.99)	
	HIV RNA (log <sub>10</sub> /ml) ≥5 vs <5	0.34	(0.05 - 2.55)	0.29	0.31	(0.04 - 2.30)	0.25	0.91	(0.35 - 2.34)	0.84
	CD4 count (/mm <sup>3</sup> )			< 0.0001			0.004			0.08
	349-200 vs ≥350	1.06	(0.37 - 2.99)		0.89	(0.33 - 2.42)		0.69	(0.25 - 1.90)	
Other cause	199-50 vs ≥350	6.09	(2.52 - 14.70)		2.35	(0.87 - 6.33)		2.50	(0.98 - 6.34)	
	<50 vs ≥350	15.87	(4.59 - 54.96)		6.55	(2.02 - 21.24)		1.52	(0.48 - 4.79)	
	HIV RNA (log <sub>10</sub> /ml) ≥5 vs <5	1.07	(0.34 - 3.35)	0.90	1.73	(0.57 - 5.23)	0.32	1.48	(0.57 - 3.83)	0.42
	CD4 count (/mm <sup>3</sup> )			< 0.0001			< 0.0001			0.02
Unknown cause	349-200 vs ≥350	3.96	(1.11 - 14.17)		1.55	(0.32 - 7.52)		1.29	(0.32 - 5.23)	
	199-50 vs ≥350	3.93	(0.70 - 22.14)		4.08	(0.85 - 19.71)		2.41	(0.55 - 10.53)	
	<50 vs ≥350	80.79	(20.60 - 316.82)		28.15	(5.55 - 142.59)		7.62	(1.78 - 32.59)	
	HIV RNA (log <sub>10</sub> /ml) ≥5 vs <5	3.19	(1.05 - 9.67)	0.04	6.60	(2.39 - 18.21)	0.0003	4.24	(1.52 - 11.87)	0.006
Other cause	CD4 count (/mm <sup>3</sup> )			0.0008			0.0003			0.005
	349-200 vs ≥350	1.89	(0.83 - 4.30)		2.63	(0.95 - 7.28)		1.45	(0.60 - 3.46)	
	199-50 vs ≥350	4.28	(1.79 - 10.22)		6.79	(2.44 - 18.93)		4.11	(1.70 - 9.96)	
	<50 vs ≥350	9.10	(2.67 - 31.02)		11.28	(3.23 - 39.40)		1.10	(0.32 - 3.82)	
Unknown cause	HIV RNA (log <sub>10</sub> /ml) ≥5 vs <5	2.90	(1.22 - 6.93)	0.02	3.74	(1.65 - 8.46)	0.001	1.28	(0.49 - 3.38)	0.61
	CD4 count (/mm <sup>3</sup> )			<0.0001			<0.0001			0.02
	349-200 vs ≥350	2.23	(1.33 - 3.73)		2.12	(1.13 - 3.98)		1.43	(0.81 - 2.53)	
	199-50 vs ≥350	5.67	(3.33 - 9.65)		4.07	(2.12 - 7.82)		2.12	(1.14 - 3.91)	
Unknown cause	<50 vs ≥350	14.48	(6.78 - 30.95)		8.65	(4.04 - 18.51)		2.63	(1.35 - 5.10)	
	HIV RNA (log <sub>10</sub> /ml) ≥5 vs <5	1.02	(0.50 - 2.08)	0.95	1.63	(1.85 - 3.19)	0.15	1.37	(0.79 - 2.37)	0.26

\* Adjusted for HIV RNA level (latest value for models first and second column, and value prior to starting cART for third column) or CD4 (if HIV RNA) and age, sex, exposure category, Hepatitis C serostatus and first line cART.

† Latest CD4 count was categorized as 349-200 and <200 cells/mm<sup>3</sup>.

HR : Hazard ratio; 95% CI : 95% confidence interval.



Table 3. Adjusted cause-specific hazard ratios of progression to death associated with cumulative time spent below 350 cells/mm<sup>3</sup>, CASCADE collaboration, 1996-2006.

	AIDS death (n=158)			Non AIDS infection (n=50)			Liver disease death (n=46)			Non AIDS cancer (n=46)			Cardiovascular disease (n=36)		
	Adj HR*	95% CI	p-value	Adj HR*	95% CI	p-value	Adj HR*	95% CI	p-value	Adj HR*	95% CI	p-value	Adj HR*	95% CI	p-value
Cumulative time spent below 350 cells/mm <sup>3</sup>															
0-1 year	1		<0.0001	1		0.007	1		0.02	1		0.17	1		0.56
1-3 years	3.01	(1.76 - 5.13)		1.59	(0.63 - 4.04)		4.48	(1.65 - 12.16)		2.17	(1.00 - 4.72)		1.06	(0.38 - 2.99)	
3-6 years	6.24	(3.65 - 10.86)		4.55	(1.87 - 11.08)		2.13	(0.68 - 6.61)		1.69	(0.69 - 4.16)		1.09	(0.38 - 3.17)	
6 years and over	8.58	(4.61 - 15.96)		3.71	(1.26 - 10.95)		3.57	(1.20 - 10.60)		2.56	(0.93 - 7.07)		1.99	(0.70 - 5.64)	
HIV RNA (log <sub>10</sub> /ml) ≥5 vs <5	8.29	(5.80 - 11.87)	<0.0001	12.71	(6.55 - 22.59)	<0.0001	2.28	(1.01 - 5.13)	0.047	2.48	(0.95 - 6.47)	0.06	4.69	(2.04 - 10.79)	0.0003

\* Adjusted for latest HIV RNA level or cumulative time below 350 CD4 cell count/mm<sup>3</sup> (if HIV RNA) and age, sex, exposure category, Hepatitis C serostatus and first line cART.

HR : Hazard ratio; 95% CI : 95% confidence interval.

Table 4. Adjusted cause-specific hazard ratios of progression to death associated with HIV RNA level according to cART treatment, CASCADE collaboration, 1996-2006.

	AIDS death (n=158)			Non AIDS infection (n=50)			Liver disease death (n=46)			Non AIDS cancer (n=46)			Cardiovascular disease (n=36)		
	Adj HR*	95% CI	p-value	Adj HR*	95% CI	p-value	Adj HR*	95% CI	p-value	Adj HR*	95% CI	p-value	Adj HR*	95% CI	p-value
HIV RNA level in log <sub>10</sub> /ml															
<5 while not on cART	1		<0.0001	1		<0.0001	1		0.06	1		0.29	1		0.007
<5 while on cART	0.43	(0.27 - 0.68)		0.35	(0.15 - 0.84)		1.02	(0.47 - 2.20)		0.89	(0.41 - 1.92)		1.34	(0.51 - 3.47)	
≥5 while not on cART	3.18	(1.80 - 5.59)		7.13	(2.87 - 17.75)		4.14	(1.33 - 12.89)		1.95 <sup>†</sup>	(0.63 - 6.07)		5.81	(1.59 - 21.24)	
≥5 while on cART	2.39	(1.36 - 4.18)		3.95	(1.49 - 10.48)		0.91	(0.22 - 3.86)		1.95 <sup>†</sup>	(0.63 - 6.07)		4.70	(1.25 - 17.73)	

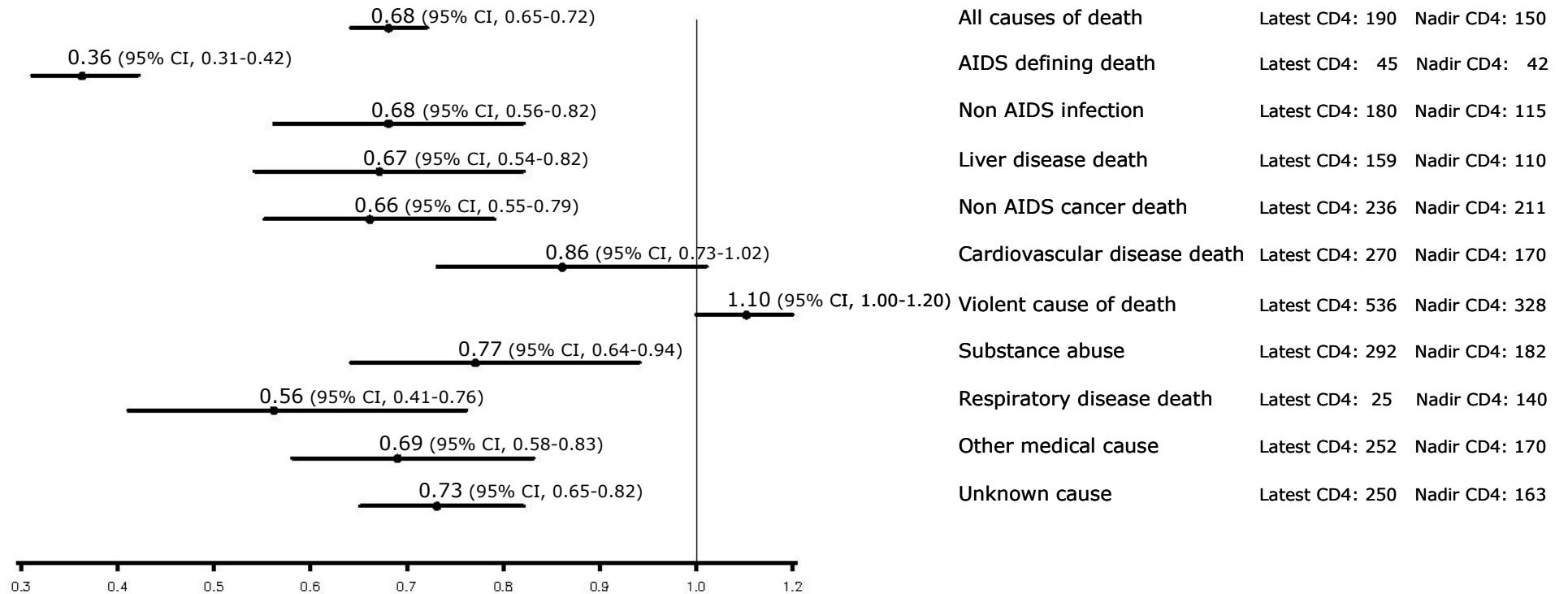
\* Adjusted for latest CD4 count, age, sex, exposure category, Hepatitis C serostatus

† Following categories : latest HIV RNA level ≥ 5 log<sub>10</sub> copies/ml while on cART or not were gathered for non AIDS cancer

HR: Adjusted Hazard Ratio, 95% CI : 95% confidence interval

Legend of figure:

Figure 1. Adjusted cause-specific hazard ratios of progression to death associated with a 100 cell increment in the latest CD4 count, CASCADE collaboration, 1996-2006.



### Adjusted Hazard Ratio (per a 100 cell increment in the latest CD4 count)

Adjusted for latest HIV RNA level and age, sex, exposure category, Hepatitis C serostatus and first line cART.

Latest CD4 : median latest CD4 cells/mm<sup>3</sup>; Nadir CD4 : median nadir CD4 cells/mm<sup>3</sup> prior to starting cART.

Figure 1

Appendix: Members of the CASCADE Collaboration are as follows:

*Steering Committee:* Julia Del Amo (Chair), Laurence Meyer (Vice Chair), Heiner C Bucher, Genevieve Chene, Deenan Pillay, Maria Prins, Magda Rosinska, Caroline Sabin, Giota Touloumi.

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